

LUCIANO VEIGA COSME

**STIMULATORY SUB-LETHAL RESPONSE OF A GENERALIST  
PREDATOR TO PERMETHRIN: HORMESIS, HORMOLIGOSIS OR  
HOMEOSTATIC REGULATION?**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

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## **BIOGRAFIA**

LUCIANO VEIGA COSME, filho de Luiz Cosme e Maria da Fátima Veiga, nasceu em Três Pontas, Minas Gerais, em 09 de Junho de 1979.

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Em junho de 2006, ingressou no curso de Mestrado em Entomologia na UFV, sob orientação do prof Raul Narciso C. Gudes. Em 2007 iniciou mestrado na Kansas State University sob orientação do professor Yoonseong Park. Atualmente, está realizando doutorado na Texas A&M University, sob orientação do prof. Michel Slotman.

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## RESUMO

COSME, Luciano Veiga. M.Sc., Universidade Federal de Viçosa, abril de 2008.  
**Resposta sub-letal estimulatória de um predador generalista à permetrina: hormese, hormoligose ou regulação homeostática?** Orientador: Raul Narciso Carvalho Guedes. Co-orientadores: Kun Yun Zhu, José Lino Neto e Marcelo Coutinho Picanço.

Historicamente a avaliação dos efeitos de pesticidas sobre artrópodes tem se baseado fundamentalmente em efeitos letais, sendo os efeitos sub-letais frequentemente negligenciadas. Entretanto, efeitos simulatórios associados com baixas doses de compostos que são tóxicos em altas doses, têm sido reportados recentemente e reconhecido como um fenômeno toxicológico. Evidência dessas respostas estimulatórias foram também verificadas em ácaros e alguns insetos-praga expostos a pesticidas, e é reconhecido como uma das potenciais causas de ressurgimento de pragas e aumento nas populações de pragas secundárias. Entretanto, parâmetros de fitness e suas implicações foram raramente considerados em estudos toxicológicos, sendo que inimigos naturais raramente são considerados nesses estudos. No presente estudo reportamos os efeitos simulatórios de dose sub-letais (variando de 0,02 a 172,00 ppb além do tratamento testemunha) do piretróide permetrina aplicado tópicamente em ninfas de terceiro instar *Podisus distinctus* (Stål) (Heteroptera: Pentatomidae). Os parâmetros estimados de tabelas de fertilidade dos insetos expostos à doses crescentes do inseticida indicaram um pequeno aumento no tempo médio de sobrevivência para as doses  $\geq 0,20$  ppb e um pico na taxa de reprodução básica na dose de 1,72 ppb. Essa tendência coincide e se correlaciona com a taxa de reprodução intrínseca da população ( $n = 18$ ,  $r = 0,78$ ,  $P = 0,0001$ ), que também apresentou um pico na dose de 1,72 ppb, levando a um valores reprodutivos maiores dos insetos expostos a estas doses. Este fenômeno foi interpretado como hormese induzida por inseticida e suas implicações são discutidas.

## ABSTRACT

COSME, Luciano Veiga. M.Sc., Universidade Federal de Viçosa, april of 2008.  
**Stimulatory sub-lethal response of a generalist predator to permethrin: hormesis, hormoligosis or homeostatic regulation?** Advisor: Raul Narciso Carvalho Guedes. Co-Advisors: Kun Yun Zhu, José Lino Neto and Marcelo Coutinho Picanço.

The assessment of pesticide effects in arthropods historically have relied heavily on acute lethal effects and the sub-lethal responses to such compounds are frequently neglected. However, stimulatory effects associated with low doses of compounds toxic at higher doses, such as pesticides, have been widely reported in recent years and recognized as a general toxicological phenomenon. Evidence of such stimulatory response has also been reported among mites and a few insect pest-species exposed to pesticides and recognized as a one of the potential causes underlying pest resurgence and secondary pest outbreaks. However, fitness parameters and its implications were seldom considered in these studies and natural enemies are not usually target of attention. Here we reported the stimulatory effect of sub-lethal doses (ranging from 0.02 to 172.00 ppb in addition to the control) of the pyrethroid permethrin topically applied to 3<sup>rd</sup> instar nymphs of the spined soldier bug *Podisus distinctus* (Stål) (Heteroptera: Pentatomidae). The parameters estimated from the fertility tables of insects exposed to the increasing doses of insecticide indicated a slight increase in the mean survival time for doses  $\geq 0.20$  ppb and a peak in the net reproductive rate at 1.72 ppb. This trend is coincident and correlated with the intrinsic rate of population growth ( $n = 18$ ,  $r = 0.78$ ,  $P = 0.0001$ ), which also shows a peak at 1.72 ppb leading to higher reproductive values of insects exposed to this dose. The phenomenon was recognized as insecticide-induced hormesis and its potential implications were discussed.

## 1. INTRODUCTION

The assessment of pesticide effects in arthropods historically has relied heavily on acute lethal effects of these toxic compounds. Pesticide bioassays with arthropods are usually designed to evaluate acute toxicity by testing individuals of a specific life stage, age and size with the primary objective of estimating their lethality through dose-response estimates employing binary scoring (i.e., dead *vs.* alive). The importance of such tests largely used for pest management and regulatory objectives is undeniable. However, the sub-lethal responses to pesticides are frequently neglected, as are more demographically-oriented studies despite their recognition as a more reliable approach to toxicity assessment (Forbes and Calow 1999, Stark and Bank 2003).

Lethal acute estimates of toxicity necessarily incur in the use of high doses of the toxic compounds in the bioassays limiting the understanding of the potential impact of low (sub-lethal) doses of these compounds. The same fact takes place throughout the toxicological scientific literature, but such reliance on high doses for toxicity and environmental impact assessments have been in intense scrutiny and controversy for the last decade (Calabrese and Baldwin 1997, 2001a, 2003a; Chapman 2001; Cook and Calabrese 2006; Murado and Vázquez 2007). Stimulatory effects associated with low doses of compounds toxic at higher doses, such as pesticides, have been widely reported in recent years and recognized as a general toxicological phenomenon (Morse 1998;



Calabrese and Baldwin 2001b, 2003ab; Calabrese 2004). Evidence of such stimulatory response has also been reported among arthropod pest-species exposed to pesticides, and has been recognized as one of the potential causes underlying pest resurgence and secondary pest outbreaks (Dittrich et al. 1974, Gerson and Cohen 1989; Hardin et al. 1995, Morse 1998, Cohen 2006). Mite pest-species, particularly the two-spotted spider mite *Tetranychus urticae*, have been the main object of attention in addition to a few other mite and insect-pest species (Dittrich et al. 1974, Carey 1982, Trichilo and Wilson 1993, James and Price 2002, Cuthbertson et al. 2003, Marcic 2003, Cutler et al. 2005). However, fitness parameters and its implications were seldom considered in these studies and natural enemies are not usually target of attention.

The recognition that the dose-response relationship of toxic compounds may be biphasic with a stimulatory response at low doses is credited to Schulz (1887), although the term hormesis was coined much later by Southam and Ehrlich (1947). The low-dose stimulation of subinhibitory doses was referred to as “hormesis” derived from the Greek “to excite” and has become widespread when describing a dose-response continuum involving a low-dose stimulation/high-dose inhibition (Calabrese and Baldwin 2001b, 2003a, Calabrese 2004). Insecticide hormoligosis is frequently used in entomology and acarology as a synonym of hormesis (Morse 1998). However, Luckey (1968) coined the term from the Greek “hormo”(“to excite”, as in hormesis) and oligo (“minute”) to describe a related phenomenon of stimulatory effects by mild levels of stress agents (not necessarily toxic) under suboptimal conditions for the target species considered, a limiting provision alien to the hormesis concept. Therefore, although hormoligosis may be considered a hormesis-related phenomenon, it does present two important distinctions from hormesis – the not necessarily toxic effect of the compound when in higher doses and the suboptimal (environmental) conditions in which the stimulatory response is expected, a frequent point of neglect in insect and mite studies. Insecticide

hormoligosis was earlier described in house crickets (*Acheta domesticus*), under suboptimal conditions of diet and temperature, when exposed to sub-lethal doses of insecticides (Luckey 1968).

Pesticide-mediated homeostatic modulation in arthropods was a term recently coined by Cohen (2006) to describe both true hormesis and hormesis-like (e.g., hormoligosis) phenomena establishing their hierarchy and allowing their proper recognition within related studies with insects and mites exposed to sub-lethal doses of pesticides, which has been faulty. Stimulatory effects observed with pesticides that are non-toxic to arthropods at high doses do not fall within the scope of hormesis and therefore cannot be considered hormetic, although the underlying mechanisms may be identical. Hence, as homeostasis in arthropods is affected and modulated by pesticides to produce stimulatory effects, the term pesticide-induce homeostatic modulation (PIHM) was suggested (Cohen 2006). Alleged hormetic effects on mites and natural enemies were the main concern in coining the PIHM definition, mainly because they are frequently non-targeted by the insecticide applications and the toxicity of the applied insecticide is not known or reported for the non-target species, compromising the recognition of hormesis as the phenomenon in place. Parallel difficulties were also reported in early surveys of the general occurrence of hormesis as a toxicological phenomenon (Calabrese 1997, 2001ab, 2003a).

Biological control agents have been rare object of attention in studies of stimulatory response to low doses of pesticides (Morse 1998, Cohen 2006). However, the phenomenon may potentially be a relevant consideration in designing pest management programs with regulatory implications. Among the natural enemies of insect-pests of recognized importance, the generalist predator *Podisus distinctus* (Stål) (Heteroptera: Pentatomidae) has broad occurrence in South America and it is commonly referred as a biological control agent of agricultural and forest insect pests relatively

tolerant to pyrethroid insecticides used against this pest species (Picanço et al. 1996, Magalhães et al. 2002, Zanuncio et al. 2002). In addition, preliminary evidence of hormesis have been reported in this species as well as in the related predator of relative minor importance *Supputius cinctipes* (Stål) (Heteroptera: Pentatomidae) (Magalhães et al. 2002, Zanuncio et al. 2003). These earlier studies did not considered overall fitness assessment in demographic studies, but rather focused on isolated fitness components.

Here we focused on fertility tables to test the hypothesis of the potential occurrence of stimulatory fitness response in the generalist predator *P. distinctus* and recognized the phenomenon taking place (i.e., if hormesis or other hormesis-related phenomenon). Forbes (2000), based on earlier surveyed studies on hormesis, suggested that the overall fitness of an organisms is unlikely to enhance at low levels of exposure to toxic agents because life-history traits are not likely to exhibit hormesis simultaneously as a result of energetic trade-offs among them. Such hypothesis has yet to be empirically tested despite the antagonist results obtained with the life table model constructed by Carey (1982) for the two-spotted spider mite.

## 2. MATERIALS AND METHODS

### 2.1 Insects and Insecticide

The bioassays were carried out using insects derived from a mass rearing colony maintained with larvae and pupae of the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in rearing chambers at controlled conditions of  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and photoperiod of 12:12 h (L:D), as described elsewhere (Magalhães et al. 2002, Zanuncio et al. 2003). Technical grade permethrin (96% pure) was provided by Syngenta Prot. Cult. (São Paulo, SP, Brazil) and acetone (p.a.) was purchased from Isofar (Jacaré, RJ, Brazil). Technical grade permethrin was diluted in acetone to obtain a broad range of doses (0.02, 0.17, 1.72, 17.20, and 172.00 ppb based on weight a.i. per wet weight of 3<sup>rd</sup> instar *P. distinctus*), in addition to a control treatment with the use of acetone only. The range of doses selected was based on preliminary tests and results of previous investigations aiming at sub-lethal doses of permethrin (Picanço et al. 1996, Magalhães et al. 2002, Zanuncio et al. 2002). Permethrin was topically applied to individual 3<sup>rd</sup> instar nymphs, stage usually recommended for field release aiming the biological control of defoliating caterpillars and other pest species (Zanuncio et al. 2002). The topical insecticide application was carried out using 1  $\mu\text{l}$  of insecticide

solution applied with a calibrated microsyringe in a microapplicator (Burkward, Rickmansworth, UK) targeting the insect scutellum.

## **2.2 Life Table Experiments and Estimated Parameters**

The fitness parameters were estimated for three batches of insects exposed to each dose of permethrin, in addition to a control (acetone only). One hundred eggs (24 h old) were used to establish the insect cohorts to be exposed to each insecticide dose. The eggs were collected from the laboratory colony using cotton wads and placed in Petri dishes (9 cm diameter x 1.5 cm high), where they were maintained with a wet cotton wad to secure high humidity and water availability to the nymphs. The emerged nymphs were transferred in groups of 20 individuals to 200 ml plastic containers with water provided in wet cotton wads. Upon reaching the 3<sup>rd</sup> instar, the nymphs were isolated in 500 ml plastic containers, where water was again provided through a wet cotton wad and pupae of yellow mealworm were daily provided *ad libitum* following Magalhães et al. (2002) and Zanuncio et al. (2003). After 24 h, the 3<sup>rd</sup> instar nymphs were individually subjected to permethrin application as previously described and subsequently placed back in their individual containers. Three groups of 20 individualized nymphs (3<sup>rd</sup> instar) were daily followed and upon reaching the adult stage, the females were paired with males subjected to the same permethrin exposure. The females were daily observed throughout their lives recording their survival, fecundity (eggs laid daily) and fertility (hatched nymphs) until eventual death.

The fertility tables were constructed for each of the three insect cohorts used in each treatment, as established from their 3<sup>rd</sup> instar onwards. The data from the egg stage until the molt to 3<sup>rd</sup> instar was common for the replicates of each treatment. The experiment was carried out in a completely randomized design and the information was used to calculate the fertility table parameters using the approximate method described by Maia et al. (2000) and following Southwood (1978).

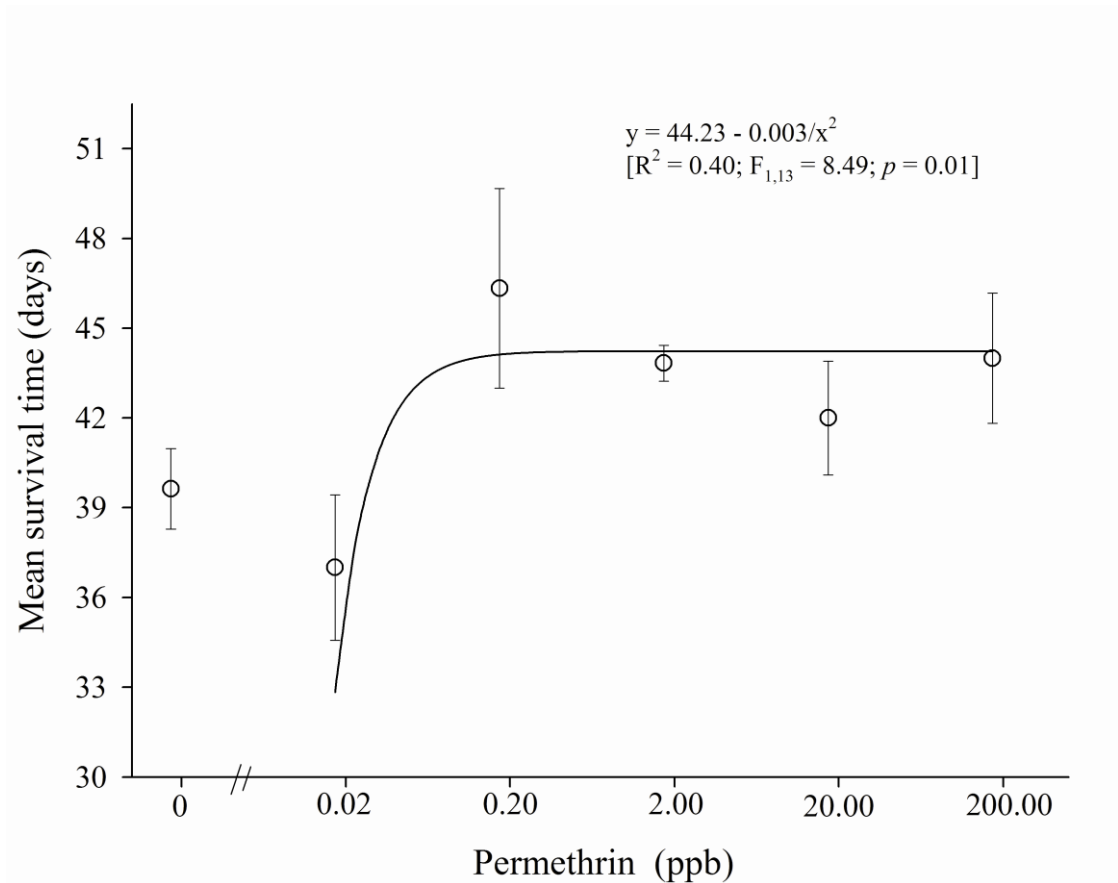
### 2.3 Statistical Analysis

The daily survivorship data was subjected to survival analysis using the procedure LIFETEST from SAS and the log-rank  $\chi^2$  to test the equality of the survival curves between doses of permethrin (SAS Institute 2002). The estimated mean survival time (days) obtained in the survival analysis, as well as the life table estimates of generation time (G), net reproductive rate (Ro), intrinsic rate of population growth ( $r_m$ ) were subjected to regression analysis using dose of permethrin as independent variable in the curve-fitting procedure from TableCurve 2D (SPSS 2000). The reproductive value of each cohort exposed to each insecticide dose was also subjected to regression analysis, but using insect age (days) as independent variable also in the curve-fitting procedure from TableCurve 2D (SPSS 2000). The models were tested from the simplest (linear and quadratic) to the alternative models of increasing complexity (non-linear peak models). The model selection was carried out based on simplicity, high F-values (and mean squares) and steep increase of  $R^2$  with model complexity. Residue distribution was also checked for each analysis on TableCurve 2D to ascertain of parametric assumptions. Punctual contrasts between the control treatment (without permethrin exposure) and the permethrin dose showing the highest response were tested using Student's t test ( $P \leq 0.05$ ).

## 3. RESULTS

### 3.1 Survival Analysis

The survival analysis of the daily survivorship data indicated significant differences between the insects exposed to increasing doses of permethrin (log-rank  $\chi^2 = 66.87$ ,  $df = 5$ ,  $P < 0.0001$ ). Therefore, the mean survival times estimated for each replicate of permethrin dose were subjected to regression analysis using the dose of permethrin as independent variable. The control treatment (0.0 ppb permethrin) was not included in the analysis to allow more flexibility in selecting the best model, since the zero value will prevent use of ratio and log-based models. The best descriptive model obtained indicated an increase in mean survival time with permethrin dose until reaching a plateau at 45 days (Fig. 1). The mean survival time of insects without permethrin exposure was significantly lower than for insets exposed to 0.2 ppb ( $t_{1,4} = 2.85$ ;  $P = 0.04$ ).



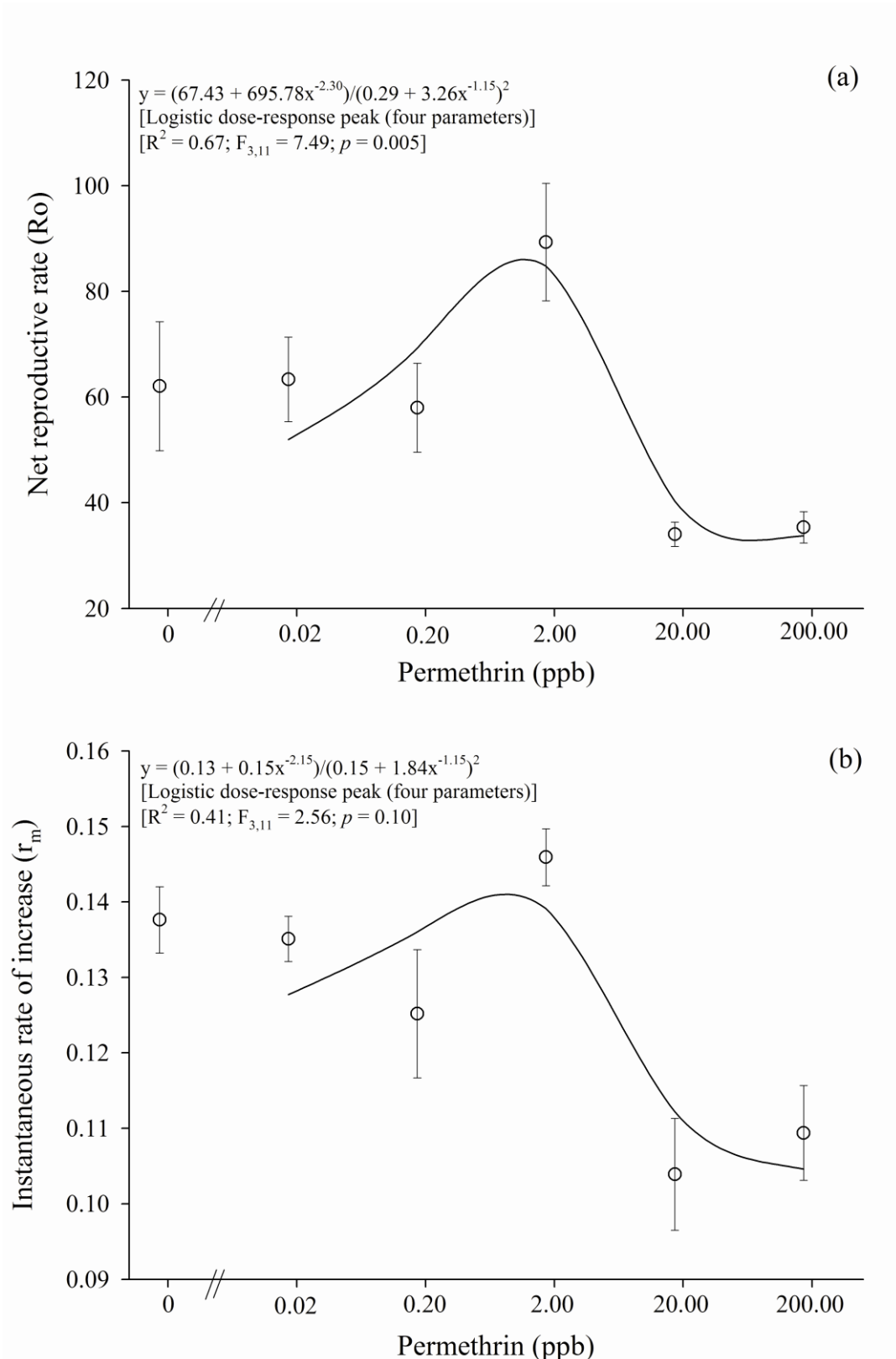
**Figure 1.** Effect of sub-lethal doses of permethrin in the mean survival time of the predator *Podisus distinctus*. Each symbol represents the mean of three replicates and the vertical bars indicate the standard error of the means.

### 3.2 Fitness parameters

The fitness parameters generation time ( $G$ ), net reproductive rate ( $R_0$ ) and intrinsic rate of population growth ( $r_m$ ) were estimated through fertility tables of females exposed to increasing doses of permethrin and subsequently subjected to regression analysis using the dose of permethrin as independent variable. As with the regression analysis of mean survival time, the estimates from the control treatment (0.0 ppb permethrin) were not included in the analysis to allow more flexibility in selecting the best model. Permethrin did not significantly affect the generation time of *P. distinctus* ( $31.78 \pm 0.80$  days;  $P > 0.30$ ). In contrast, permethrin significantly affected the net



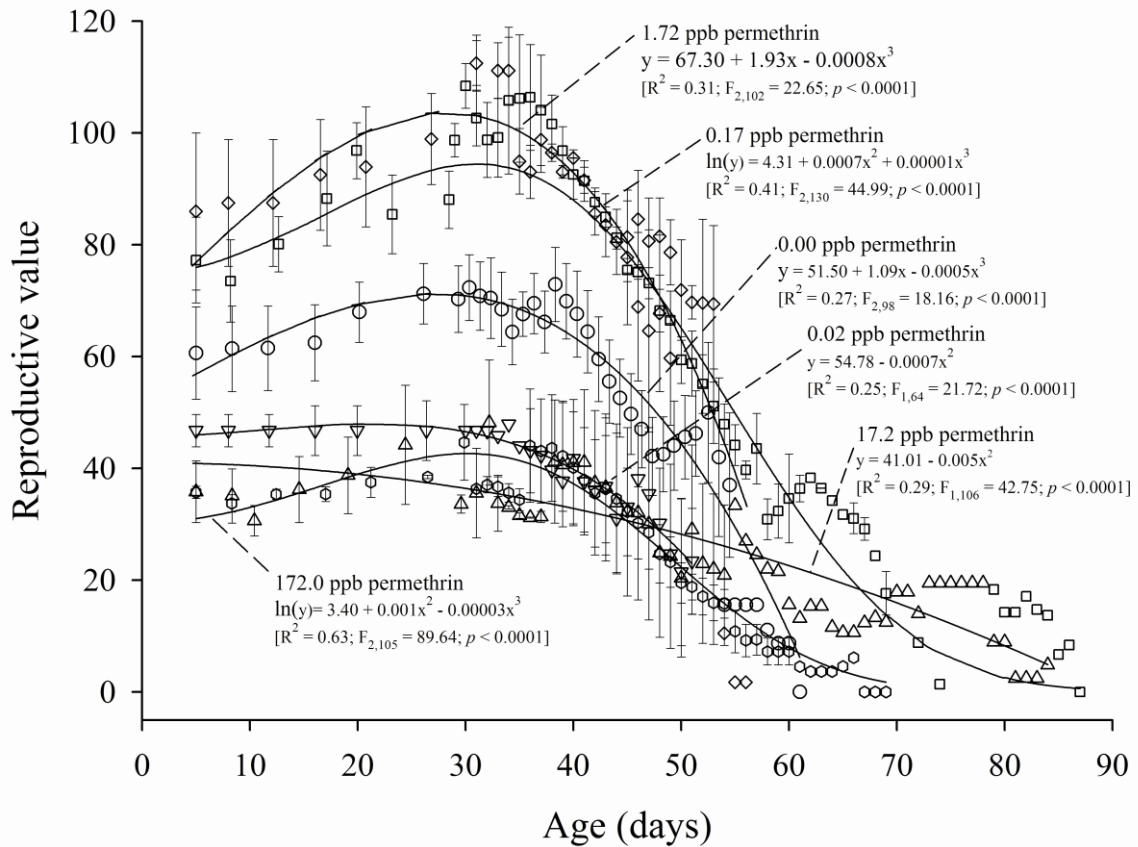
reproductive rate, which showed a peak at 1.7 ppb permethrin ( $89.33 \pm 11.11$ ; Fig. 2a). This peak in net reproductive rate was significantly different from the net reproductive rate without permethrin exposure ( $62.06 \pm 9.02$ ) ( $t_{1,4} = 2.73$ ;  $P = 0.05$ ). The intrinsic rate of population growth was significantly correlated with the net reproductive rate ( $n = 18$ ,  $r = 0.78$ ,  $P < 0.0001$ ) and showed a trend similar to this parameter, although significant only at  $P = 0.10$  (Fig. 2b). This was due to the opposite action of generation time reducing the impact of the reproductive rate in the intrinsic rate of population growth, whose peak also at 1.7 ppb permethrin ( $0.146 \pm 0.004$ ) did not differ significantly from the rate of population growth of insects not exposed to permethrin ( $0.138 \pm 0.004$ ) ( $t_{1,4} = 0.90$ ;  $P = 0.40$ ).



**Figure 2.** Effect of sub-lethal doses of permethrin in the net reproductive rate (a) and intrinsic rate of population growth (b) of the predator *Podisus distinctus*. Each symbol represents the mean of three replicates and the vertical bars indicate the standard error of the means.

### 3.3 Reproductive Value

The regressions carried out with the estimated reproductive values of insects exposed to increased doses of permethrin indicated a peak around 30 days of age and again the females exposed to sub-lethal doses of the insecticide in the range of 0.2 and 1.7 ppb exhibited higher reproductive values, followed by the non-exposed insects and then the females exposed to the remaining doses (Fig. 3).



**Fig. 3.** Age-dependent reproductive value of the predator *Podisus distinctus* exposed to increasing doses of permethrin. Each symbol represents the mean of three replicates and the vertical bars indicate the standard error of the means.

## 4. DISCUSSION

A stressor is an external agent (e.g., heat, pesticide) that results in a physiological response from an organism required to maintain its homeostasis. Homeostatic modulation of a given organism may be manifested as stimulatory changes in life history traits and if the stressor is a pesticide, the pesticide-mediated (or induced) homeostatic modulation may lead to stimulatory effects on reproduction (Cohen 2006). The suggested concept is broad enough to accommodate hormesis and related phenomena, and have been consistently reported in studies with both phytophagous and predatory mite species exposed to pesticides (Dittrich et al. 1974, Carey 1982, Gerson and Cohen 1989, Trichilo and Wilson 1993, James and Price 2002, Cuthbertson et al. 2003, Marcic 2003). The phenomenon has also been reported in some insect pest species (e.g., Penman and Chapman 1980, Morse and Zareh 1991, Ferguson and Chapman 1993, Cohen 2006), and a few predatory arthropods (e.g., Yokoyama and Pritchard 1984, Toft and Jensen 1998, Cutler et al. 2005). Here we also recognized a permethrin-induced homeostatic modulation in the predator *Podisus distinctus*, confirming the preliminary suggestion by Magalhães et al. (2002), since the reproductive output of this spined soldier bug increased with sub-lethal doses of this pyrethroid.

Hormoligosis, together with hormesis and other hormesis-related phenomena, are examples of pesticide-mediated homeostatic modulation (Cohen 2006). Hormoligosis was first reported by Luckey (1968) in house crickets (*Acheta domesticus* (L.)) exposed to sub-lethal doses of a range of different insecticides (mainly organochlorines, but also organophosphates), which were the different stressors used, under suboptimal conditions of temperature and nutrition. The conditions of this experiment satisfy the hormesis criteria – a sub-lethal dose of a toxic compound and assessment of a parameter (weight gain in the case) able to display either stimulatory or inhibitory responses (Calabrese and Baldwin 2001ab, 2003a) – were satisfied and this is a likely reason for its frequent confusion with hormesis within entomology and acarology (see Morse 1998, Cohen 2006). However, hormoligosis was defined based on the use of a stressor (which is not necessarily a toxic compound within an applied framework) under suboptimal conditions for the targeted species (Luckey 1968). The suboptimal conditions are systematically neglected in the studies reporting pesticide hormoligosis, and sometimes toxicity of the pesticide under investigation to the species is not considered as pointed by Cohen (2006). The hormesis criteria is not met if the pesticide considered is selective in favor of a non-target species (e.g. a natural enemy or another non-target species) and if its toxicity to the studied species is not indicated somehow, but the hormoligosis concept is satisfied since the pesticide is still a stressor.

Here we used optimal conditions for rearing the predator species *P. distinctus* (Zanuncio et al. 2002). This predator, although tolerant to pyrethroids in general as other related species (Picanço et al. 1996, Magalhães et al. 2002, Zanuncio et al. 2002), is affected by higher doses of this compound even in the sub-lethal range used in the present study. The toxic effects of permethrin to this species are perceptible at 17 ppb, where significant decrease of reproductive output takes place, and there is significant increase in mortality for permethrin doses above 2 ppm (data not presented). Therefore,

the experimental conditions and results obtained clearly indicate that the hormesis phenomenon takes place in *P. distinctus*. Furthermore, the insecticide-induced hormesis in *P. distinctus* was detected using demographic parameters (net reproductive rate and consequently reproductive value), which we are not aware of for any other insect predator species.

The use of fitness parameters as the toxicological endpoint in our study allows us to test the prediction of Forbes (2000). Based on earlier surveyed studies on hormesis, Forbes (2000) suggested that the overall fitness of an organism is unlikely to enhance at low levels of exposure to toxic agents because life-history traits are not likely to exhibit hormesis simultaneously as a result of energetic trade-offs among them. Some evidence to the contrary exists concerning pesticide-mediated homeostatic modulation in two-spotted spider mites (not hormesis) (Dittrich et al. 1974, Carey 1982), but such evolutionary expectation has yet to be tested for pesticide-induced hormesis in arthropods. *Podisus distinctus* exhibits increased reproductive output under sub-lethal doses of permethrin and such increase was within the 30-60% range of increase commonly reported for hormesis (Calabrese 2003a). However, when the intrinsic rate of population growth ( $r_m$ ) was considered in our study, the increase observed under sub-lethal doses of permethrin was barely significant and certainly undistinguishable from the control without permethrin exposure. Such finding lay credence to the prediction by Forbes (2000) and the suggestion that reproduction may be favored under sub-lethal doses of toxic compounds at the expense of other physiological process (e.g., maintenance), as predicted by the principle of resource allocation put forward by Sibly and Calow (1986). However, the same may not take place with other organisms and conditions, what deserves attention.

Regardless of the evolutionary implications of pesticide-induced hormesis and related phenomena, they are potentially important for the design of pest management

programs. It is only potentially important because we do not know how widespread it is and how their occurrence in pest-natural enemy systems will mediate such interaction. We suspect though that in the system explored here, hormesis is unlikely to have substantial impact in the field performance of *P. distinctus* against its prey species in forest and agriculture settings because of the modest increase in the reproductive output observed and the lack of significant impact in the predator fitness reflected in its rate of population growth. However, more realistic experimental settings will be important to properly test this hypothesis.

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